REMARKS

In this response to the Office Action dated February 20, 2009, claim 1 has been amended to broaden the claimed subject matter to use of both MT-I and MT-II. As discussed below, both isoforms have substantially the same activity. Additionally, the claims have been amended to clarify that outgrowth of both forms of neurites, including axons and dendrites, can occur as a result of performing the claimed method. Finally, the claim has been amended to clarify that a sufficient amount of said metallothionein is delivered to stimulate said outgrowth of neurites.

A number of dependent claims have been amended to correct minor informalities, some of which arose based on the amendments to Claim 1. In addition, Claims 28 and 29 have been newly added. As discussed below, these amendments are fully supported by the specification as filed and do not add any new matter. Claims 1, 3-17, 28, and 29 are currently pending in the application. In view of the amendments and remarks as set forth herein, Applicants respectively request withdrawal of the claim rejections and reconsideration of the pending claims.

Discussion of claim amendments

Support for the amendments to claim 1 can be found from the specification as originally filed. As expressly disclosed in page 4, lines 6-10 of the specification, the claimed method includes direct contacting as well as indirect contacting of a solution of metallothionein isoform with a target tissue. Therefore, the removal of "direct" from claim 1 is supported. In addition, the specification described in page 3, lines 12-14, 17-18, and page 4, lines 1-5, that the metallothionein in the present invention may be selected from any one or a combination of known metallothionein classes including MT-I, MT-II, MT-III, MT-IV, and the associated informs as well as a synthetic metallothionein, which comprises features of one or more isoforms. Moreover, Example 4 in the specification experimentally showed the activity of MT-I and MT-II combination in neurite outgrowth. Therefore, the recitation in claim 1 related to the metallothionein isoforms is fully supported. The features of claim 1 regarding neurite outgrowth via delivery of sufficient amount of metallothionein isoform(s) can be found from the embodiments illustrated in the Examples 1-4 and Figures 1-17 of the specification, which tested the effect of metallothionein on neurite formation, elongation, and outgrowth. Support for new claims 28 and 29 can also be found from the Examples 1-4 and related figures and descriptions

As noted, the claim amendments in this response are fully supported and do not constitute any new matter. Applicants respectfully request entry of these amendments and reconsideration of the pending claims.

Claim rejections by Penkowa under 35 U.S.C 102(b) or 35 U.S.C. 103(a)

Claims 1 and 4 were rejected under 35 U.S.C. 102(b) as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Penkowa *et al.* (2002, Journal of Comparative Neurology 444(2): 174-189), as evidenced by Sigma M4952 and Garrett (2000, The Prostate 43: 125-135). Applicants respectfully traverse these rejections.

In the previous response filed on January 18, 2008, Applicants explained that recovery of injured nerve system (e.g. CNS) is composed of multiple, discrete processes as follows.

A. Injury → **B.** Protection and survival of neurons → **C.** Regenerative growth → **D.**Reconnection of neural network

As previously explained, the present invention is related to the final steps **C** and **D** of regenerative growth and reconnection of the neural network based on outgrowth of neurites. In contrast, the Penkowa et al. reference is directed to step **B**, the protection and survival of neurons following injury.

Steps B and C, neuroprotection and neuroregeneration, respectively, are particularly distinct and fundamentally different processes in many respects. Neuroprotection refers to survival of cells or reducing damage to cells, thereby preventing death of neurons. In contrast, neuroregeneration refers to regrowth and repair of cells after damage to cells has occurred. One simple way of distinguishing them is that they occur at different times after the initial injury. Death of neurons (i.e. the process which is blocked by neuroprotectants) occurs 1-2 days after injury. Regeneration of neurons, however, does not occur until 4-7 days after injury. Also the way to detect neuroprotection and neuroregeneration is completely different. For example, cell death such as apoptosis or necrosis shall be measured to monitor neuroprotection, whereas direct observation of neurons and/or immunohistochemical detection of growth associated proteins shall be applied to detect neuroregeneration.

Penkowa teaches the protective role of metallothioneins in CNS during neurological degeneration. *See* Abstract of Penkowa. This reference, however, explores only the neuroprotective capacity of metallothionein and regenerative growth of neurons is not examined at all. Penkowa does not even cite any techniques that would allow detection of

regenerative growth of neuron. As noted above, neuroregeneration occurs at later stage and cannot be detected with a method designed for neuroprotection. Therefore, Penkowa, which is exclusively restricted to neuroprotection, cannot and does not teach a claimed activity of metallothionein in neuroregeneration.

Furthermore, it is not possible to predict or conclude that an agent promotes neuroregenerative growth, based on prior knowledge of its neuroprotective capacity. In fact there are many neuroprotective agents, which do not have neuroregenerative properties (e.g. glutamate receptor antagonists such as MK-801), known in the art. Therefore, one with ordinary skill in the art, who would be aware of the foregoing notion as well as Penkowa, would not consider the neuroregenerative roles of metallothioneins in light of Penkowa.

Despite the obvious discrepancy between Penkowa and the instant application, the Examiner appears to reason that the administration of metallothionein according to Penkowa would inherently show the neuroregenerative activity as cited in the pending claims. However, this conclusion is not accurate, as an insufficient amount of metallothionein would be delivered to stimulate said outgrowth of neurites.

In order to evidence that based on the teachings of Pankowa et al., one would not provide sufficient metallothioneien to stimulate outgrowth of neurites, Applicants are providing experimental data showing that the Penkowa method is unable to stimulate neurite outgrowth. *See* the attached Declaration and accompanying Exhibits A and B. In the experiment described in the Declaration, Applicants tested whether the Penkowa method can deliver a substantial amount of metallothionein that is required to stimulate the outgrowth of neurites. Applicants administered metallothionein in a 50 fold higher dose than as originally stated in Penkowa to mice under the conditions as described by the reference. The presence and/or level of metallothionein in the mice brain was monitored by two separate methods, namely, immunohistochemistry and western blotting. As clearly seen in the images of Exhibit A and B, no metallothionein was detected in the brain regardless of the detection methods. Especially with the detection by the western blotting, the negative result indicates that the actually delivered amount of metallothionein by Penkowa method would be much less than 0.016 μg/g tissue wet weight. In contrast, as disclosed in the Examples 1-2 of the specification, the claimed method delivers a substantial amount (e.g. 1 to 10 μg/ml for the specific test condition of the Examples) of metallothionein to the injured rat cortex and stimulates neurite formation, elongation and outgrowth. These contradicting data indicate that the administration method of Penkowa, which actually used 50 fold less than tested herein, is

incapable of delivering any substantial amount of metallothionein to the neurites as required to regenerate the neurites according to claim 1.

As noted above, Penkowa does not teach or suggest the claimed method of stimulating neurite outgrowth by administering metallothionein. Moreover, it is proven that the Penkowa method is unable to stimulate neurite outgrowth as claimed in the present invention. Thus, nothing in Pankowa et al. provides any disclosure of the delivery of a sufficient amount of metallothionein to stimulate neurite outgrowth, as recited by the presently pending claims. Therefore, the claimed method of claims 1 and 4 is neither anticipated nor obvious over Penkowa, even as evidenced by Sigma M4952 and Garrett. Accordingly, withdrawal of the claim rejections over Penkowa is respectfully requested.

Claim rejections by Giralt under 35 U.S.C 102(b) or 35 U.S.C. 103(a)

Claims 1, 4, and 17 were rejected under 35 U.S.C. 102(b) as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Giralt *et al.* (2002, Experimental Neurology 173: 114-128). Applicants respectfully traverse these rejections.

Like Penkowa, Giralt also teaches the neuroprotective capacity of metallothionein, but not its role in regenerative growth. As noted, it is impossible to predict the neuroregenerative activity of an agent such as metallothionein based on its neuroprotective capacity. Therefore, Giralt cannot and does not teach a claimed activity of metallothionein in neuroregeneration.

Further, the Giralt method was also tested by Applicants and proven that it is unable to stimulate neurite outgrowth as recited in claim 1. *See* the attached Declaration and Exhibits. As done to Penkowa method, a 50-fold higher dose than as originally stated in Giralt was administered to mice as described in Giralt, however no noticeable level of metallothionein was detected in the neuronal tissue (i.e. Brain).

As such, the claimed method of claim 1 is neither anticipated norobvious over Giralt. Accordingly, withdrawal of the claim rejection over Giralt is respectfully requested. As to claims 4 and 17, they incorporate all the limitations of claim 1, to which they refer. Therefore, claims 4 and 17 are patentable over Giralt for at least same reasons that claim 1 is patentable. Applicants respectfully request reconsideration of claims 4 and 17 in light of the patentability of claim 1.

Claim rejections over Penkowa under 35 U.S.C. 103(a)

Claims 1, 4, and 6-12 were rejected under 35 U.S.C. 103(a) as being unpatentable over Penkowa. As described previously, Penkowa fails to disclose the subject method of claim 1. For example, Penkowa is exclusively restricted to neuroprotection and does not consider the neuroregeneration stage at all in its reference. Therefore, Penkowa is unrelated to a claimed method that is directed to neuroregeneration. Further, even if Penkowa method is practiced with an excess amount of metallothionein to the neuroregeneration stage, it cannot deliver a substantial amount of metallothionein to stimulate the neurite as cited in claim 1. *See* the attached Declaration and Exhibits. Therefore, Penkowa does not and cannot render the subject method of claim 1 obvious. Accordingly, claim 1 is patentable over Penkowa and its dependent claims 4 and 6-12 are also in condition of allowability. Reconsideration of the claim rejections is respectfully requested.

Claim rejections over Penkowa and FR 2813529 under 35 U.S.C. 103(a)

The Examiner maintained the rejections to claims 1-12 under 35 U.S.C. 103(a) as being unpatentable over Penkowa in view of FR 2813529 ('529 patent). Applicants respectively traverse the rejections.

As noted, Penkowa is deficient to render the claimed method obvious. As discussed aboved, Penkowa is unable to administer sufficient metallothionein to stimulate neurite outgrowth as recited in claim 1. Further, '529 patent does not cure this deficiency of Penkowa. For example, as correctly cited by the Examiner, the '529 publication fails to teach administering the composition such that target neurons or neuronal areas are exposed to the metallothionein-containing composition. *See* page 6, lines 8-9 of the Office Action. As such, even if Penkowa and '529 patent could be properly combined, the combination would still fail to lead one having ordinary skill in the art to the claimed method of claim 1. As such, Applicants respectfully request withdrawal of the rejection to claim 1 over the references. Claim 2-12, which depend from claim 1, are also in condition of allowability in light of claim 1 being patentable. Reconsideration of claims 2-12 is respectfully requested.

Claim rejections over Penkowa, Deguchi, and Yoshimura under 35 U.S.C. 103(a)

Claims 1, 4, and 6-13 were rejected under 35 U.S.C. 103(a) as being unpatentable over Penkowa in view of Deguchi (2000, Pharmaceutical Research 17(1): 63-69) and Yoshimura

(2001, Proc Natl Acad Sci USA 98(10): 5874-5879), which is a new ground of rejection. Applicants respectively traverse the rejection.

As noted, Penkowa fails to teach or suggest several features of claim 1. Further, Deguchi and Yoshimura do not cure the deficiency of Penkowa. Deguchi and Yoshimura are related to basic fibroblast growth protein (bFGF) and its delivery to the brain. The Examiner asserted that the references by Deguchi and Yoshimura would suggest an injection of proteins directly into the brain and thereby, in combination with Penkowa, rendering the claimed method obvious. Applicants respectfully disagree with this assertion.

Even if Penkowa, Deguchi and Yoshimura could be properly combined, the combination would not lead one having ordinary skill in the art to the method according to claim 1. Penkowa focuses on only the neuroprotection stage, which is not at all overlapping with the neuroregeneration stage addressed by the presently claimed invention. Deguchi and Yoshimura are not related to neuroregeneration at all. Therefore, the combination of the references would lead one having ordinary skill in the art only to administer metallothionein to address the neuroprotection stage following injury. Nothing in any of the references would suggest outgrowth of neurons as claimed in claim 1. As such, even if one skilled in the art would combine the teachings of the references in the manner suggested by the Examiner, such a person would only deliver the metallothionein in the immediate stages following injury to achieve neuroprotection. As noted above, the neural regeneration and reconnection of neural network occur at a much later stage following injury. Nothing in the combination of references would lead one having ordinary skill in the art to administer metallothionein at the proper stage following injury to achieve the neurite outgrowth recited in the claim. Accordingly, Claim 1 is patentable over the combination of Penkowa, Deguchi and Yoshimura. Claims 4, and 6-13, which are dependent claims from claim 1, are also patentable for at least the same reason that claim 1 is patentable as well as in view of their own features. Applicants respectfully request reconsideration of the claim rejections over the references.

Claim rejections over Penkowa, Deguchi, Yoshimura, and Asanuma under 35 U.S.C. 103(a)

Claims 1, 4, 6-13, and 15 were rejected under 35 U.S.C. 103(a) as being unpatentable over Penkowa in view of Deguchi, Yoshimura, and in further view of Asanuma (2002, Neurosciences Letters 327:61-65). Applicants respectively traverse the rejection.

As noted in the foregoing section, claim 1 is patentable over Penkowa, Deguchi and Yoshimura. Further, Asanuma does not remedy the deficiencies of the cited references. Asanuma teaches the roles of metallothionein on dopaminergic neurotoxicity of 6-hydroxydopamine, however does not disclose and suggest their role in neuroregeneration or neurite outgrowth at all. Therefore, even Asanuma is combined with Penkowa, Deguchi and Yoshimura, the method rendered from this combination still fails to disclose a method of stimulating neurite outgrowth as in claim 1. Therefore, Applicants respectfully request withdrawal of the rejections to claim 1 and the dependent claims 4, 6-13, and 15 over the cited references.

Claim rejections by Penkowa, Degucchi, Yoshimura, and Walsh under U.S.C. 103(a)

Claims 1, 4, 6-14, and 16 were rejected under 35 U.S.C. 103(a) as being unpatentable over Penkowa in view of Deguchi, Yoshimura, and in further view of Walsh (US Patent Application publication 2002/0155170). Applicants respectively traverse the rejections.

As noted above, claims 1, 4, and 6-13 are patentable over Penkowa, Deguchi and Yoshimura. Further, Walsh does not remedy the deficiencies of Penkowa, Deguchi and Yoshimura. Walsh teaches the implication of metallothionein in Alzheimer's disease, however does not disclose their role in neuroregeneration at all. Furthermore, as correctly cited by the Examiner, Walsh does not disclose administering metallothionein for not only treating its target disease, Alzheimer's disease but also stimulating neurite outgrowth. *See* page 9, lines 17-18 of the Office Action. Therefore, even Walsh is combined with Penkowa, Deguchi and Yoshimura, it still fails to disclose the claimed method of claims 1, 4, and 6-13. Therefore, Applicants respectfully request withdrawal of the claim rejections to claims 1, 4, and 6-13. Claim 16, which is dependent claim of claim 1, should be also in condition of allowability in light of claim 1 being patentable over the cited references. Reconsideration of claim 16 is respectfully requested.

Allowability of new claims 28 and 29

New claims 28 and 29 are added and their support can be found, for example, from the Examples 1-4 and related figures and descriptions. Therefore no new matter is added. Theses claims, through their dependency from claim 1, incorporate all the features of claim 1. As discussed above, claim 1 is patentable over all the cited references. Therefore, new claims 28

and 29 are also patentable at least for the same reasons that claim 1 is patentable. Applicants respectfully request consideration of new claims 28 and 29 for the patentability.

No Disclaimers or Disavowals

Although the present communication may include alterations to the application or claims, or characterizations of claim scope or referenced art, Applicants are not conceding in this application that previously pending claims are not patentable over the cited references. Rather, any alterations or characterizations are being made to facilitate expeditious prosecution of this application. Applicants reserve the right to pursue at a later date any previously pending or other broader or narrower claims that capture any subject matter supported by the present disclosure, including subject matter found to be specifically disclaimed herein or by any prior prosecution. Accordingly, reviewers of this or any parent, child or related prosecution history shall not reasonably infer that Applicants have made any disclaimers or disavowals of any subject matter supported by the present application.

CONCLUSION

Applicants have endeavored to address all of the Examiner's concerns as expressed in the outstanding Office Action. Accordingly, arguments in support of the patentability of the pending claim set are presented above.

In light of the above remarks, reconsideration and withdrawal of the outstanding rejections is respectfully requested. If the Examiner has any questions which may be answered by telephone, he is invited to call the undersigned directly.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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Exhibit A

Immunohistochemistry

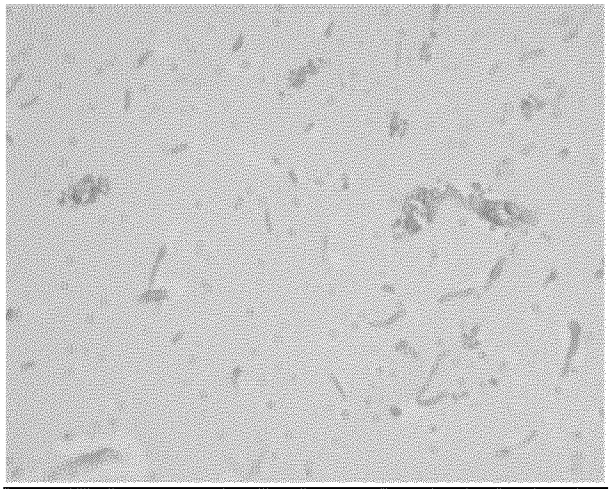

Figure A: Immunohistochemistry of transgenic mouse brain in vicinity of cryolesion, 40 minutes after administration of 250 µg/10g body weight Zn-MT-2A by intraperitoneal route. Note absence of immunoreactivity around or within neural cells, or vascular cells. Control sections are available (contralateral side, no antibody controls).

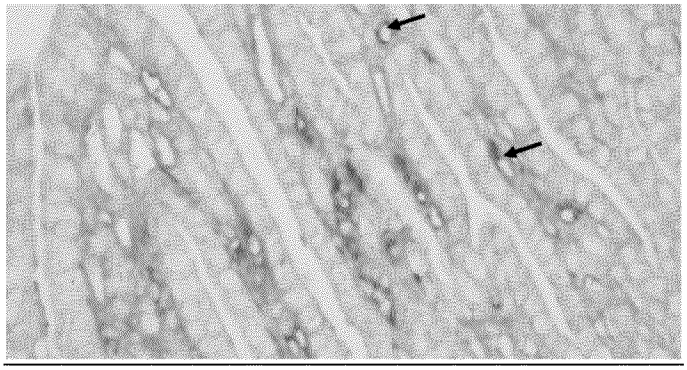

Figure B: In comparison, a section of transgenic mouse kidney 4 days after injection of 100 µg/10g body weight Zn-MT-2A by intraperitoneal route. Note the clear immunoreactivity (shown as dark areas) in the capillaries beneath the endothelium (arrows).

Exhibit B

Western Blotting

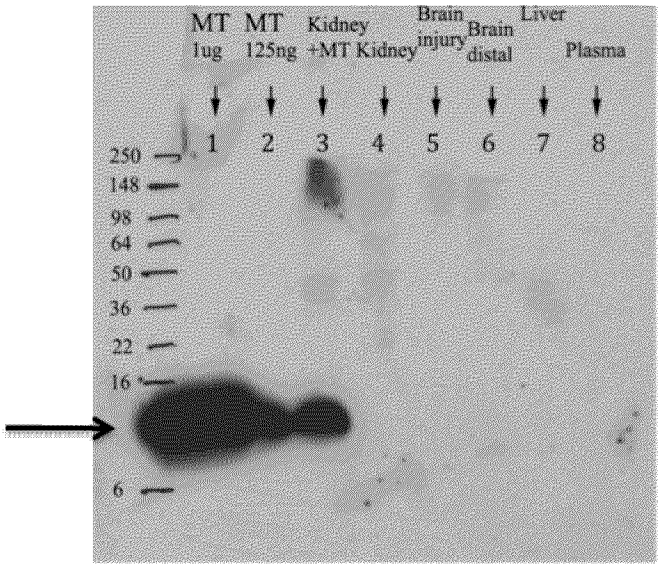

Figure 2. Western blot. Transgenic mouse brain cryolesions were performed concurrently with metallothionein administration by intraperitoneal route. Kidney (25 µg lysate track 4), brain (injury site, 10 µg lysate, track 5), brain (25 µg lysate, track 6), liver (25 µg lysate, track 7) and plasma (25 µg lysate, track 8) collected and processed for western blotting. In addition, control samples MT (1 µg, track1), (125 ng, track 2), and kidney (25 µg lysate from a kidney spiked with 1 µg MT, track 3) were included.

All controls (lanes 1-3) showed the expected outcome (Arrow Fig 2: a band of metallothionein at about 12 kD, the apparent size at which this protein migrates on SDS-PAGE), but in contrast no metallothionein was observed in any mouse tissue, including brain (track 6) and brain lesion (track 5), as a consequence of intraperitoneal administration. We routinely perform this technique and have shown that its lowest detection limit is 8 ng of metallothionein. We can therefore infer, by reference to this limit of detection, that metallothionein levels at the site of the lesion following intraperitoneal administration at 50 times the levels used by Giralt and Penkowa, must be substantially below 0.8 µg /g tissue wet weight. That is, the approach used by Giralt and Penkowa could not have resulted in metallothionein levels in the brain sufficient to promote neurite outgrowth.